Volume 208, number 2 FEBS 4221 November 1986

Turnover of rat liver ornithine transcarbamylase

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Received 12 September 1986

The relative half-life of ornithine transcarbamylase from rat liver has been determined using the double isotope technique and affinity chromatography. The calculated half-life (6–9 days) is similar to that of mitochondria and of the other mitochondrial enzyme of the urea cycle, carbamoyl-phosphate synthase. Therefore, both mitochondrial urea cycle enzymes are most probably degraded mainly via the lysosomal (autophagic) pathway of mitochondrial protein degradation.

Protein turnover *Mitochondria* *Lysosome* *Ornithine carbamoyltransferase*

1. INTRODUCTION

Ornithine transcarbamylase (ornithine carbamoyltransferase, EC 2.1.3.3) is the second enzyme of the urea cycle and is found, almost exclusively, in liver mitochondria of ureotelic animals [1]. This enzyme is a good candidate for the study of the turnover of proteins because its synthesis (on membrane-free polysomes in the form of a larger precursor with an NH_2-terminal extension), transport into the mitochondrial matrix (with a half-life of 1–2 min) and processing to the mature enzyme (apparently coincident with the transport), have been extensively studied (see [2,3] and references cited therein). However, very little is known concerning the degradation of the enzyme.

Ornithine transcarbamylase is a very stable enzyme; its initial postulation was based on its separation from carbamoyl-phosphate synthase I, the other mitochondrial enzyme of the urea cycle, on the basis of the high stability of ornithine transcarbamylase to heat [4]. Nevertheless, ornithine transcarbamylase is susceptible to a number of proteolytic enzymes, including lysosomal proteases, particularly in the presence of ATP [5]. Nicoletti et al. [6], using a double isotope technique, estimated the half-life of carbamoyl-phosphate synthase I to be 7.7 days. Such studies on rat liver ornithine transcarbamylase have not been documented. Thus, it appeared of interest to assess its half-life in rat liver in relation to other cell compartments and to compare it with other rat liver mitochondrial enzymes, in particular carbamoyl-phosphate synthase.

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Abbreviations: δ-PALO, δ-*N*-(phosphonoacetyl)-L-ornithine; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; $t_{1/2}$, half-lives; k_d, rate constant of degradation

2. MATERIALS AND METHODS

2.1. *Affinity chromatography of rat liver ornithine transcarbamylase*

δ-PALO was prepared and further purified by paper chromatography [7]. The purified δ-PALO was then coupled to epoxy activated Sepharose 6B [7]. Rat liver ornithine transcarbamylase was isolated by a batch method [7]. Mitochondria [8] were diluted with 10 mM Tris-HCl, pH 7.4, containing 1 mM EDTA and 0.5% Triton X-100 to a protein concentration of about 10 mg/ml, homogenized briefly and sonicated (MSE sonicator, top capacity, 3 × 5 s). After 10 min centrifugation at 15000 × *g* and 4°C, the supernatant

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00145793/86/$3.50

was mixed with 10 g of δ-PALO-Sepharose and was incubated for 30 min in a shaking bath at 27°C. The mixture was filtered on a sintered glass funnel and was then washed with 100 ml of 10 mM Tris-HCl, pH 7.4, 1 mM dithiothreitol, and with 100 ml of 10 mM Tris-HCl, pH 7.4, 1 mM dithiothreitol, 100 mM KCl.

Suction was discontinued and 10 ml of 10 mM carbamoyl phosphate was added, incubated for 5 min, and the eluant collected by suction. Incubation with carbamoyl phosphate was repeated once, eluants were combined, mixed with ammonium sulfate (463 g/l), and the ornithine transcarbamylase was collected by centrifugation and dissolved in 100 mM phosphate buffer, pH 7.8, 1 mM EDTA, 1 mM dithiothreitol.

2.2. *Measurement of enzyme degradation*

The double isotope method [9] was used. Rats were injected intraperitoneally on day 0 with [^{14}C]leucine (25 μCi and 0.083 μmol leucine). Injections with [^{3}H]leucine (75 μCi and 0.083 μmol leucine) were performed at appropriate time intervals (3, 6 and 10 days). For zero time, rats were injected with both isotopes at the same time.

Rats were starved overnight before all injections, and otherwise maintained on a normal protein diet and water ad libitum. Rats were killed 4 h after injection with [^{3}H]leucine. Three rat livers were processed, separately, simultaneously. The livers were removed to ice cold 250 mM sucrose, and all the following steps carried out at 4°C. Mitochondria were separated [8]. Microsomes and cytosol were prepared from the mitochondrial supernatant by centrifugation at 100000 × *g* for 60 min. All fractions were stored at −20°C.

2.3. *General*

Ornithine transcarbamylase activity was determined as in [10]. SDS-PAGE (10% acrylamide) was done in slab gels according to Laemmli [11]. Protein was determined using a biuret-deoxycholate assay [12].

3. RESULTS AND DISCUSSION

To 10 g of Sepharose 0.56 mmol of δ-PALO could be coupled. This compares well with the results obtained by De Martinis et al. [7]. As can be seen in fig.1, δ-PALO (purified by paper chromatography) inhibited ornithine transcarbamylase to the same extent as reported by Mori et al. [13].

From rat liver homogenate, 70–80% of the original ornithine transcarbamylase was recovered with a specific activity of ~280 units/mg protein. This specific activity is similar to or even better than the specific activities of ornithine transcarbamylase purified by similar methods [7,14–16]. When the carbamoyl phosphate eluate (see section 2) was precipitated with trichloroacetic acid and characterized by SDS-PAGE, it was found that ornithine transcarbamylase, as identified by molecular mass standards and purified rat liver ornithine transcarbamylase, constituted, by and large, the major part of the protein (fig.2). Minor contaminating bands appeared only in gels prepared with enzyme that had been stored for more than 1 week at 4°C without addition of ammonium sulfate.

In spite of the interest in protein turnover, there are very few published values for individual proteins, likely due to the fact that there are few reliable methods to measure it. The double isotope procedure [9] has been used successfully to determine the turnover of liver proteins [17], including mitochondrial proteins [6,18]. Although it is an ex-

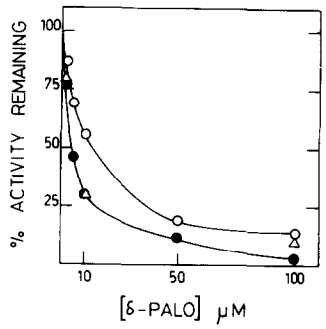

Fig.1. Inhibition of ornithine transcarbamylase by δ-PALO. Purified beef liver ornithine transcarbamylase was incubated at 37°C for 10 min using the conditions of Mori et al. [13]. Incubation mixtures contained in 500 μl, 25 μmol Hepes, pH 7.2, ~43 μU of ornithine transcarbamylase, 2.5 μmol L-ornithine, 2.5 μmol carbamoyl phosphate and the indicated quantities of δ-PALO. (○) δ-PALO before purification (contaminated with alfa-PALO), (●) δ-PALO after purification by paper chromatography, (△) values reported by Mori et al. [13].

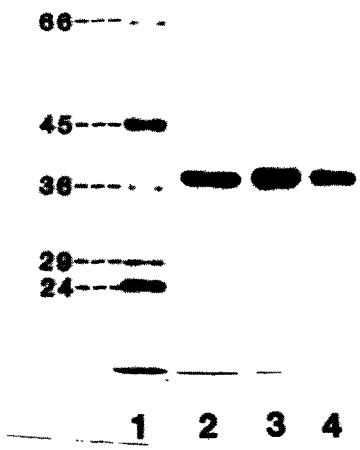

Fig.2. SDS-PAGE (10% acrylamide) of ornithine transcarbamylase. Lanes: 1, molecular mass standards; 2, purified rat liver ornithine transcarbamylase; 3 and 4, rat liver ornithine transcarbamylase isolated by affinity chromatography from dual labeled mitochondria (20 and 15 μg of protein, respectively).

pensive procedure and requires considerable technical skill, it has several advantages over other procedures, particularly its reliability and rapidity. Selection of this procedure was also based on the fact that most of the published half-life values have been obtained with the double isotope procedure, thus allowing comparisons.

Using $^3H/^{14}C$ ratios obtained for liver fractions, standard curves were plotted, and used to determine the rate constants of degradation (k_d values) of isolated ornithine transcarbamylase from their respective $^3H/^{14}C$ ratios. The relative half-lives ($t_{1/2}$) were calculated from the relationship $t_{1/2} = 0.693/k_d$. From table 1, we estimated the half-life of ornithine transcarbamylase to be about the same as for mitochondria, between 6 and 9 days. Since this procedure is best applied to proteins with a half-life longer than 2 days, we found the calculated half-life to be within the applicable limits of the procedure.

Our value for the half-life of rat liver ornithine transcarbamylase is a measure of the longest half-lives for rat liver mitochondrial proteins [19]. This value is close to the half-life obtained by Nicoletti et al. [6] for the rat liver carbamoyl-phosphate synthase (7.7 days) which is also present in the mitochondrial matrix [20] and is also preferentially located in periportal areas [21] in the liver acinus. Interestingly, it differs, however, from the half-life obtained in the same work for glutamate dehydrogenase (1 day) [6], which appears partially associated to the inner mitochondrial membrane [22] and is mainly found in intermediate and pericentral areas of the hepatic acinus [23]. It should be noted that the half-life values obtained for carbamoyl-phosphate synthase and ornithine transcarbamylase in another species, frog liver, are also very similar, 3.5 days and 4 days, respectively [1]. Therefore, since the half-lives of both mitochondrial urea cycle enzymes agree well with the estimated whole mitochondrial protein turnover, it appears that these two enzymes, which represent more than one fifth of the total mitochondrial matrix protein, are degraded mainly via the lysosomal (autophagic) destruction of mitochondria.

Table 1

Isotope ratios, k_d values and half-lives ($t_{1/2}$) of rat liver fractions

Fraction	3-day interval			6-day interval			10-day interval		
	$^3H/^{14}C$	k_d	$t_{1/2}$	$^3H/^{14}C$	k_d	$t_{1/2}$	$^3H/^{14}C$	k_d	$t_{1/2}$
Homogenate	5.0 ± 0.9	0.20	3.5	5.9 ± 0.4	0.17	4.1	7.1 ± 0.4	0.18	3.9
Mitochondria	3.9 ± 0.9	0.12	5.8	4.4 ± 0.2	0.09	7.7	5.1 ± 0.4	0.10	6.9
Microsomes	5.7 ± 1.1	0.26	2.7	7.5 ± 0.1	0.23	3.0	9.1 ± 0.1	0.24	2.9
Cytosol	4.8 ± 1.1	0.18	3.9	5.6 ± 0.4	0.15	4.6	6.8 ± 0.3	0.17	4.1
OTC	4.0 ± 0.3	0.12	5.8	4.1 ± 0.1	0.08	8.7	4.8 ± 0.4	0.08	8.7

Fractions were prepared as described in section 2. k_d values were taken from standard curves prepared as described in the text. Half-lives were calculated from k_d values. Values are given as $(days)^{-1}$ for k_d and days for $t_{1/2}$. Each value represents the average from 2–3 rats. OTC, ornithine transcarbamylase

ACKNOWLEDGEMENTS

Supported in part by CAICYT, FISS, Comité Conjunto Hispano-Norteamericano and the IIC-KUMC International Molecular Cytology Program.

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